METHODS FOR CONTROLLING PESTS USING RNAI

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional application of, and claims priority to U.S. patent application Ser. No. 11/992,091 filed on May 8, 2008 which is a national stage filing under 35 U.S.C. §371 of International Application No. PCT/IB2006/004008, filed on Sep. 18, 2006, which claims benefit of 60/837,910, filed on Aug. 16, 2006, and claims benefit of 60/771,160, filed on Feb. 7, 2006, and claims benefit of 60/758,191 filed on Jan. 12, 2006, and claims benefit of 60/718,034, filed on Sep. 16, 2005, the contents of each of which are herein incorporated by reference in their entireties.

SEQUENCE LISTING

[0002] A Sequence Listing in ASCII text format, submitted under 37 CFR §1.821, entitled "80386_SEQLIST_ST25.txt", 774 kilobytes in size, generated on Sep. 11, 2014 and filed via EFS-Web is provided in lieu of a paper copy. This sequence listing is hereby incorporated by reference into the specification for its disclosures.

FIELD OF THE INVENTION

[0003] The present invention relates generally to genetic control of pest infestations. More specifically, the present invention relates to recombinant technologies for repressing or inhibiting expression of target coding sequences in a pest.

INTRODUCTION

[0004] Insect and other pests can cause injury and even death by their bites or stings. Additionally, many pests transmit bacteria and other pathogens that cause diseases. For example, mosquitoes transmit pathogens that cause malaria, yellow fever, encephalitis, and other diseases. The bubonic plague, or black death, is caused by bacteria that infect rats and other rodents. Compositions for controlling microscopic pest infestations have been provided in the form of antibiotic, antiviral, and antifungal compositions. Methods for controlling infestations by pests, such as nematodes and insects, have typically been in the form of chemical compositions that are applied to surfaces on which pests reside, or administered to infested animals in the form of pellets, powders, tablets, pastes, or capsules.

[0005] Commercial crops are often the targets of insect attack. Substantial progress has been made in the last a few decades towards developing more efficient methods and compositions for controlling insect infestations in plants. Chemical pesticides have been very effective in eradicating pest infestations. However, there are several disadvantages to using chemical pesticides. Not only are they potentially detrimental to the environment, but chemical pesticides are not selective and can pose harm to non-target flora and fauna. Chemical pesticides persist in the environment and generally are slow to be metabolized, if at all. They accumulate in the food chain, and particularly in the higher predator species. Accumulation of chemical pesticides results in the development of resistance to the agents and in species higher up the evolutionary ladder, they can act as mutagens and/or carcinogens and cause irreversible and deleterious genetic modifications.

[0006] Because of the dangers associated with chemical pesticides, biological approaches have been developed for controlling pest infestations. For example, biological control using protein Cry3A from *Bacillus thuringiensis* have effectively controlled Colorado potato beetle larvae either as formulations sprayed onto the foliage or expressed in the leaves of potatoes. An alternative biological agent is double stranded RNA (dsRNA). Over the last few years, downregulation of genes (also referred to as "gene silencing") in multicellular organisms by means of RNA interference has become a well-established technique.

[0007] RNA Interference (RNAi) provides a potentially powerful tool for controlling gene expression because of its specificity of target selection and remarkably high efficiency in target mRNA suppression. RNAi refers to the process of sequence-specific post-transcriptional gene silencing mediated by short interfering RNAs (siRNAs) (Zamore, P. et al., *Cell* 101:25-33 (2000); Fire, A. et al., *Nature* 391:806 (1998); Hamilton et al., *Science* 286, 950-951 (1999); Lin et al., *Nature* 402:128-129 (1999)). While the mechanics underlying RNAi are not fully characterized, it is thought that the presence of dsRNA in cells triggers RNAi by activating the ribonuclease III enzyme Dicer (Zamore, P. et al., (2000); Hammond et al., *Nature* 404, 293 (2000)). Dicer processes the dsRNA into short pieces called short interfering RNAs (siRNAs), which are about 21 to about 23 nucleotides long and comprise about 19 base pair duplexes (Zamore et al., (2000); Elbashir et al., *Genes Dev.*, 15, 188 (2001)). Following delivery into cells, the siRNA molecules associate with an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which brings together the antisense strand of the siRNA and the cellular mRNA gene target. RISC cleaves the mRNA, which is then released and degraded. Importantly, RISC is then capable of degrading additional copies of the target mRNA.

[0008] Accordingly, the present invention provides methods and compositions for controlling pest infestation by repressing, delaying, or otherwise reducing gene expression within a particular pest.

SUMMARY OF THE INVENTION

[0009] In one aspect, the invention provides an isolated polynucleotide sequence comprising a nucleic acid sequence set forth in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 49-158, 159, 160, 163, 168, 173, 178, 183, 188, 193, 198, 203, 208, 215, 220, 225, 230, 247, 249, 251, 253, 255, 257, 259, 275-472, 473, 478, 483, 488, 493, 498, 503, 513, 515, 517, 519, 521, 533-575, 576, 581, 586, 591, 596, 601, 603, 605, 607, 609, 621-767, 768, 773, 778, 783, 788, 793, 795, 797, 799, 801, 813-862, 863, 868, 873, 878, 883, 888, 890, 892, 894, 896, 908-1040, 1041, 1046, 1051, 1056, 1061, 1071, 1073, 1075, 1077, 1079, 1081, 1083, 1085, 1087, 1089, 1091, 1093, 1095, 1097, 1099, 1101, 1103, 1105, 1107, 1109, 1111, 1113, 1161-1571, 1572, 1577, 1582, 1587, 1592, 1597, 1602, 1607, 1612, 1617, 1622, 1627, 1632, 1637, 1642, 1647, 1652, 1657, 1662, 1667, 1672, 1677, 1682, 1684, 1686, 1688, 1690, 1692, 1694, 1696, 1698, 1700, 1702, 1704, 1730-2039, 2040, 2045, 2050, 2055, 2060, 2065, 2070, 2075, 2080, 2085, 2090, 2095, 2100, 2102, 2104, 2106, 2108, 2120-2338, 2339, 2344, 2349, 2354, 2359, 2364, 2366, 2368, 2370, 2372, 2384-2460, 2461, 2466, 2471, 2476 and 2481. In one embodiment, a double stranded ribonucleotide sequence is produced from the expression of a polynucleotide sequence, wherein contact of said ribonucleotide sequence by a pest inhibits the growth